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AND REPRODUCTIVE SUCCESS IN
ANDIFLORUM AND AQUILEGIA CAERULEA

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EFFECTS OF SIMULATED ACID RAIN ON POLLEN
GERMINATION AND REPRODUCTIVE SUCCESS IN
ERYTHRONIUM GRANDIFLORUM AND AQUILEGIA CAERULEA

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Abstract. Experiments were designed to determine the impact of acid rain on plant reproductive processes, a critical component of a species' life history. Research was carried out in herbaceous alpine communities at the U.S.D.A. Forest Service Glacier Lakes Ecosystem Experiments Site in the Snowy Mts. of Wyoming. Pollen from Erythronium grandiflorum and Aquilegia caerulea was assayed in vitro to determine its sensitivity to acid during germination and growth. Both species demonstrated reduced in vitro pollen germination in acidified media, but Erythronium pollen demonstrated greater tolerance to low levels of acidity than Aquilegia pollen. Field pollinations were carried out in Erythronium grandiflorum and Aquilegia caerulea to determine the reproductive success of plants exposed to simulated ambient precipitation (pH 5.6) or simulated acid precipitation (pH 3.5) prior to pollination. In Erythronium, no differences were observed in seed set and seed weight of fruits resulting from the two pollination treatments. In Aquilegia, fruits resulting the acid spray treatment produced fewer seeds and lighter seeds.

Key words: acid rain; pollen germination; pollination; alpine plants; Erythronium grandiflorum, Aquilegia caerulea

Topic phrases: effects of acid rain on seed set in alpine plants; effects of acidity on pollen germination in vitro; pollen selection in natural plant communities

INTRODUCTION

The alteration of atmospheric processes resulting from human activity has become an area of increasing concern in the scientific and political arena (Davis, 1988, Defries and Malone, 1989, Hutchinson and Meena, 1987, Mooney et al, 1987, Pitelka and Raynal, 1989). Plants exhibit a variety of responses to air pollution, ranging from severe physiological disruption in very sensitive individuals to barely perceptible changes in very tolerant plants (Berry, 1973; Davis and Wood, 1972; Evans and Curry, 1979; Houston, 1974). The evolutionary survival of all species depends on the ability to grow and reproduce under prevailing environmental conditions.

Most studies of acid rain effects on plants have focused on direct or indirect injury to vegetative tissues in species of importance to agriculture or forestry (Ferenbaugh, 1976; Hindawi et al, 1980; Evans et al, 1978; Lee et al, 1981). Acid rain can also have indirect and direct effects on plant reproduction. Reproductive output may suffer as an indirect consequence of physiological disruption such as chloroplast damage (Ferenbaugh, 1976, Hindawi et al 1980) and root degeneration (Klein, 1984) since reduced photosynthesis and transpiration is likely to affect flower production and fruit development. These effects may underlie the seed yield reductions observed in some crop species under acid stress (Evans et al, 1981). Additional indirect effects

of acid rain on plant reproduction include the effects of acidity on animals that function as pollinators, seed dispersers and predators (Alstad and Edmunds, 1982). Direct effects of acid rain on plant reproduction may involve damage to floral tissues and developing seeds, and disruption of fertilization through effects on stylar receptivity, pollen viability, germination, and tube growth. Pollen germination is very sensitive to changes in pH, and several researchers have reported disruption of pollen growth in response to acidity, primarily in agricultural species (Cox, 1986; Sidhu, 1983; Wertheim and Craker, 1988; Von Ryan et al, 1986).

Our experiments examine the effects of acid rain on reproductive processes in a natural plant community. Our research site is at an elevation of 3300 meters in the Snowy Mountains of southeast Wyoming. The site lies within the Medicine Bow National Forest, and ~~it~~ has been designated the Glacier Lakes Ecosystem Experiments Site (GLEES). GLEES is the focus of an interdisciplinary research program carried out by scientists from the Rocky Mountain Forest and Range Experiment Station and a group of university collaborators. The primary thrust of research at GLEES is to identify the effects of global atmospheric changes on this alpine ecosystem. These experiments focus on the direct effects of acidity on pollen germination and reproductive success in alpine plants under field conditions.

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The Glacier Lakes Ecosystem Experiments Site is a 300 hectare glacial cirque basin that includes three lakes and the entire watershed surrounding them. The site receives a mean annual snowfall of 2 meters and generally remains snow covered from September through June. Scattered snowfields persist into the month of August. GLEES is also a National Atmospheric Deposition Program site. In addition to ~~these~~ ^{precipitation} data, a variety of hydrological and meteorological variables such as soil ^{water} chemistry, ^{stream and lake chemistry,} solar radiation, relative humidity, and air and soil temperature are directly monitored at the site. The landscape patterns at GLEES are diverse, including rock and scree slopes, krummholz, coniferous forest and alpine meadow. ^{Vascular plant species and cover have been monitored at 100 permanent vegetation plots at the GLEES}

This study focused on two species of montane and subalpine plants that are abundant at GLEES, glacier lily, Erythronium grandiflorum Pursh (Liliaceae) and columbine, Aquilegia caerulea James (Ranunculaceae). Erythronium is a herbaceous perennial that emerges from an underground corm just as the snow is melting (mid-June to mid-August depending on elevation). Flower buds are developing at the time of emergence, and plants generally produce one or two large (2-3 cm) flowers during the season. The flowers contain six yellow tepals and two whorls of three exerted anthers around a central three-lobed style. The reproductive biology and pollination ecology of Erythronium grandiflorum has been extensively studied by Cruzan (Cruzan 1989; Cruzan 1990 a,b) and by Thomson and coworkers in Colorado populations (Thomson, 1986; Thomson, Price, Waser and Stratton, 1986; Thomson and Thomson,

1989; Thomson, 1989) Bumblebees (Bombus occidentalis Greene and Bombus bifarius Cresson) are the principal pollinators in the Colorado populations.

Aquilegia caerulea is a rhizomatous perennial herb of the central and southern Rocky Mountains of North America, commonly found at elevations of 2,000 to 3,600 meters. The populations at GLEES flower from early July to late August, and produce variable numbers of flowers depending on plant size, generally ranging from 5-20 flowers throughout the season. Flowers are large (5-6 cm) and erect with a central column of numerous styles surrounded by numerous stamens. The five petaloid sepals alternate with five petals that extend into long (4-5 cm) nectar spurs. The GLEES populations exhibit white petal blades and blue sepals and spurs; this variety has been labelled Aquilegia caerulea var. caerulea (Munz, 1946). Miller (1978,1981) describes a variety of insect visitors to Aquilegia caerulea populations in the vicinity of Gothic, Colorado, but his studies indicate that bumblebees (Bombus occidentalis Greene) and hawkmoths (Hyles lineatus (Fabricius)) are the principle pollinators.

MATERIALS AND METHODS

Two sets of experiments were carried out at GLEES during the months of June through August 1989. The first group of experiments were designed to study the effects of acidity on

pollen germination in vitro in Erythronium grandiflorum and Aquilegia caerulea. The other set of experiments involved hand pollinations to compare the effects of acidity on in vivo reproductive processes in these two species.

Preliminary germination tests in vitro were carried out on freshly collected pollen from Erythronium and Aquilegia populations at GLEES. We used standard Brewbaker Kwack media (Brewbaker and Kwack, 1963) with a 0.29 M osmoticum (Polyethylene glycol, M.W 400). Germination of Aquilegia pollen was also enhanced by addition of 2mg/L Tris (Tris hydroxyaminomethane) to the media (Roberts et al, 1983); pH of all medias used for screening was held constant at pH 5.6. The viability of pollen from both species was assayed using Alexander's stain (Alexander, 1969).

We acidified the standard Brewbaker Kwack media with a 1:1 (w/w) solution of nitric and sulfuric acid to create experimental medias with pH values of 2.5, 3.5, 4.5, 5.5 and 6.5. Seventy five microliters of each experimental germination media was placed in a depression slide, and 100 - 200 pollen grains of each species were added by lightly contacting the surface of the media with a newly-dehisced anther while looking through a dissecting microscope. The slides were placed on filter paper saturated with distilled water in a closed Petri dish to maintain high humidity. Slides inside these Petri dish "germination chambers" were incubated for two hours at 25 degrees C in a laboratory oven. Samples were

examined and photographed under a light microscope, the number of germinated pollen grains was counted, and the condition of pollen tubes in each media was noted. We also carried out a series of trials in which we measured the pH of the media before and after adding pollen to determine the influence of pollen addition on the pH of the media. In these trials one fresh anther of each species was added to 1 ml of each experimental germination media in a plastic multiple well tray. The pH in each well was measured successively for a continuous monitoring period of 2-3 hours.

A series of hand pollinations were carried out in Erythronium grandiflorum and Aquilegia caerulea to examine the effects of acid rain on reproductive processes in vivo. Flowers of each species were destamated, tagged and covered with cotton mesh pollinator-exclusion bags. An artificial rain solution was prepared using background ion concentrations obtained from NADP data from ^{the}GLEES in 1987 and 1988 (Table 1). We adjusted the artificial rain with a 1:1 (w/w) solution of sulfuric and nitric acid to create an "ambient rain" simulant (pH 5.6) and an "acid rain" simulant (pH 3.5). Stigmas of all flowers were misted with either acid rain or ambient rain simulant from hand held spray bottles. The intensity and duration of each spraying was standardized and calculated to result in approximately 4 ml of fluid applied to each flower.

Hand pollinations were then carried out by brushing fresh anthers across each stigmatic surface to assure abundant pollen deposition. In Erythronium, pollinations were carried out 1-2 hours after stigmas were sprayed. In Aquilegia pollinations were carried out approximately 24 hours after stigmas were sprayed. In Erythronium, non-sprayed plants in the same population served as pollen donors. In Aquilegia, out-cross pollen was obtained from non-sprayed flowers on adjacent experimental plants. When possible, crosses were made between the same seed parent and pollen donor in both the acid rain and ambient rain treatment; these are referred to as "matched crosses". After pollination, the plants were covered again with pollinator-exclusion bags, and the fruits were collected when mature. The seeds from each fruit were counted and weighed. Mature fruits were also collected from naturally pollinated Aquilegia and Erythronium flowers that had not been sprayed, and seeds were counted and weighed.

The effect of acid rain on vegetative tissues of Erythronium grandiflorum was also examined. Leaves of 42 plants were sprayed daily for 9 days with our "acid rain" simulant (pH 3.5) and leaves of an additional 42 plants were sprayed daily for 9 days with our "ambient rain" simulant (pH 5.6). The intensity and duration of each spraying was standardized and calculated to result in approximately 4 ml fluid applied to each leaf. Leaves were collected after 10 days for evaluation of the extent of tissue damage in the two treatments.

RESULTS

Significant variation in germination percentages of pollen growing in vitro at different pH values was found in both species studied (Table 2). Both species showed a marked decrease in germination percentage between pH 4.5 and pH 3.5. The majority of tubes that formed in media below pH 4.5 were irregular and coiled. Pollen of both species failed to germinate below pH 2.5.

Aquilegia pollen showed greater sensitivity at pH 3.5 (1 % germination) than pollen from Erythronium (30% germination). Pollen from both species changed the pH of the germination media (Table 2), although larger changes were seen in media with Erythronium pollen, which tended to decrease the pH of experimental medias at pH 6.5 and 5.5, and increase the pH of medias at pH 2.5, 3.5 and 4.5.

The effects of simulated acid rain on reproductive processes in vivo differed in the two species investigated. In Erythronium there was no difference in the number or weight of seeds produced by plants in the ambient or acid spray treatment (Table 3). There was also no difference in the number or proportion of aborted seeds. In addition, the seed set of hand pollinated plants under both spray treatments was significantly greater than the seed set of non-sprayed plants under natural pollination conditions. No visible damage was found on Erythronium leaves sprayed with acid or ambient rain simulant. There were scattered necrotic areas on

a few leaves from both treatments, but damage was too slight in both treatments to warrant measurement of leaf damage areas.

In Aquilegia, flowers sprayed with acid rain simulant produced significantly fewer seeds and significantly lighter seeds than flowers in the ambient spray treatment (Table 4). These differences were particularly striking in matched crosses (Table 5). Fruits from the acid spray treatment also produced greater proportion of aborted or unfertilized seeds per fruit (Table 4 and 5). Flowers from both spray treatments produced fewer seeds than non-sprayed naturally pollinated flowers from the experimental plant population, although the difference was greater between fruits from the acid treatment. Non-sprayed naturally-pollinated flowers produced seeds that were heavier than seeds from the acid spray treatment, but lighter than those from the ambient spray treatment.

DISCUSSION

Differences in the acid sensitivity of Erythronium and Aquilegia pollen germinating in vitro were seen at pH levels below 4.5, suggesting that these two species may respond differently to atmospheric changes resulting in precipitation below this pH level. The pH of ambient rainfall measured at the GLEES site ranged from pH 4.6 - 5.5 during the flowering season in 1988 and 1989 (NADP data), so these experimental results do not indicate

any danger to the species studied under current levels of acid pollution. However, pH values within the range shown to be inhibitory to Erythronium and Aquilegia (pH 3.5-4.5) are common in much of the northeastern United States and parts of Europe (Wellburn, 1988).

Although pollen from both species is capable of changing the pH of media, a range of distinct pH levels were maintained in these experiments. Aquilegia pollen raised the pH of medias at all initial pH levels, although these pH changes were generally small. The effect of Aquilegia pollen on the pH of media used in this experiment may not reflect the maximum buffering ability of Aquilegia pollen, due to the presence of Tris in this media. Larger pH changes were measured in medias with Erythronium pollen which did not contain Tris. The direction of pH change in medias containing Erythronium pollen suggests that pollen of this species is capable of altering the pH of its environment in the direction of maximum pollen germination (5.0 - 5.5). A comprehensive study of pollen buffering ability in herbaceous species at GLEES is currently underway, since ^{our} preliminary studies ~~we have done~~ indicate that there is variation among species with regard to this ability.

The results of our in vitro sensitivity tests in Erythronium and Aquilegia compare well with data obtained by Cox (1986) from other species. Cox studied the pH sensitivity of pollen growing in vitro in a group of twelve species from various habitats in

eastern Canada. He found that pollen from broadleaf trees and understory flora (Oenothera parvifolia, Maianthemum canadense, Trillium grandiflorum, Diervilla lonicera, Prunus pensylvanica) was more sensitive to low pH than pollen from the conifers sampled. Cox reported inhibition thresholds for pollen from most broadleaf trees and understory flora to be pH 3.6 - 4.6, while conifers had thresholds ranging from pH 2.6 - 4.6 or 2.6 - 3.6). Results from a broad survey of pollen sensitivity in additional herbaceous species at GLEES indicates a general decrease in pollen germination at pH 3.5, although the strength of the inhibition varies among species (McKenna, in preparation)

The seed set data from the pollination studies indicates that reproductive processes in vivo in Erythronium are less sensitive to acidity than reproductive processes in Aquilegia. This result corresponds with the differences in sensitivity seen in pollen germination studies in vitro. Lower seed set of Aquilegia flowers sprayed with acid rain simulant may reflect poor pollen growth in vivo; this possibility is currently being explored through fluorescence microscopy studies. Microscopic studies of Erythronium styles pollinated after acid and ambient spray treatments indicate a reduced number of pollen tubes in styles that received an acid rain treatment (McKenna, in preparation). The reduction seen in Erythronium pollen germination in vitro at pH 3.5 may not indicate the likelihood of decreased fertilization under natural conditions, since a 30% germination rate may still allow full seed set. Any factor that results in a decreased

germination percentage will reduce the range of potential parental genotypes however, and a reduced opportunity for competition between pollen grains could result in lower average offspring fitness (Bertin, 1990; McKenna and Mulcahy, 1983; McKenna, 1986; Mulcahy, 1979; Snow, 1983).

A possible explanation for the difference in the seed set of these two species following the acid spray treatment may relate to differences in phenology and habitat. Erythronium is one of the first species to flower; developing buds emerge from snowfields as they are melting. In contrast, Aquilegia is found on well drained soils of rocky ledges and blooms later in the season. Erythronium may experience a more acidic environment since snow chemistry studies at GLEES indicate that snow ^{accumulates} concentrates airborne pollutants ~~in insoluble form~~ ^{are released from the snow pack} that ~~become soluble as the snow melts~~ ^{to the} releasing a pulse of acidity (D.L. Fox, personal communication). Thus the difference in these two species' relative sensitivity to acidity may reflect selection for survival in different environments.

Hand pollinations in Erythronium following both acid and ambient spray treatments resulted in greater numbers of seeds per fruit than found in open pollinated non-sprayed controls. This suggests that reproduction may be limited by the availability of pollen under natural conditions. Casual observation of pollinator activity indicates that a depauperate pollinator fauna may be at least partially responsible for the relatively lower seed sets in

controls. In Aquilegia, open pollinated non-sprayed controls produced greater numbers of seeds than hand pollination of either the acid or ambient sprayed flowers. In this case, both spray treatments may have interfered with the fertilization process. It is also possible that ovule number may vary throughout the season in this species. Since the open pollinated flowers were pollinated later than the hand pollinated flowers, the differences in seed number may reflect increased ovule number in flowers formed during this period. Future studies including ovule counts and hand pollinations of non-sprayed flowers have been designed to address these questions.

The long term effects of differential sensitivity to pH change among these alpine species can only be explored through experiments designed to evaluate the plants throughout the entire growing season. Funk and Bonde (1986) reported a differential reproductive response in field populations of Acomastylis rossii and Bistorta vivipara sprayed with nitric/sulfuric acid mist. Flower production was reduced in Acomastylis, while Bistorta showed an increase in flower production and a decrease in asexual bulblet production. A long term study on the effects of simulated acid rain treatments on Erythronium and Aquilegia is currently underway at the GLEES site.

Alpine plant communities are diverse, reflecting the wide diversity of microhabitats in the alpine environment, ranging from exposed scree slopes to wet meadows (Bliss, 1971). Complex

ecological relationships exist between alpine plant species and other biotic and abiotic features of ~~of~~ the alpine ecosystem (Tranquillini, 1964). Human alteration of the atmospheric component of this ecosystem is likely to result in widespread impacts on these relationships. ^{Plants in alpine communities are the ATET} The results of our studies on the ^{low} sensitivity of reproductive processes in alpine plants to acidity ^{in the} suggest that acid rain may produce significant impacts on plant ^{because of} reproduction in the alpine community, and that the response to ^{acidic} acidity varies among species. The effects of acid rain on plant ^{rain} reproduction may be subtle, but prolonged reproductive losses in ^{stress} plants could lead to long term community alteration, including changes in species composition and diversity. For example, a shift in species composition following the removal of sensitive species may alter competitive interactions (Materna, 1984; Smith, 1974) and disrupt plant interactions with soil microorganisms such as nitrogen fixing bacteria (Shriner and Johnston, 1981; Strayer et al, 1981) and pathogenic or mutualistic fungi (Shriner, 1978; Bruck et al, 1981; Klein, 1984). The long term response of plants to acid rain may also depend on interactions with other stress factors such as drought, frost, predators, pathogens, and nutrient stress.

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TABLE 1. NADP - GLEES Precipitation Chemistry (June 1988, July and August 1987). Values are mg/L.

	JUNE (n=3)	JULY (n=4)	AUGUST (n=4)	Y	s	n
PH	4.77	5.06	5.01	4.97	0.252	11
Ca	0.32	0.17	0.11	0.189	0.123	11
Mg	0.038	0.023	0.019	0.026	0.015	11
K	0.033	0.015	0.011	0.018	0.020	11
Na	0.094	0.047	0.071	0.069	0.033	11
NH	0.063	0.10	0.011	0.084	0.052	11
NO	1.57	0.70	0.83	0.984	0.416	11
Cl	0.10	0.08	0.10	0.102	0.037	11
SO	1.44	0.64	0.73	0.893	0.518	11

TABLE 2. Mean pollen germination percentages of each species at each pH level. Values in parentheses are the mean number of grains per sample. Final pH values are based on measurements taken after 2 hours in a separate pollen-buffering study. Kruskal-Wallace test was used to compare germination percentages within each species.

ERYTHRONIUM GRANDIFLORUM (BK - PEG Media); N = 14

<u>Initial pH</u>	<u>Final pH</u>	<u>% Germination</u>	Test	
			<u>Statistic</u>	<u>p</u>
6.5	5.7	69.3 (139)	84.58	.001
5.5	5.3	64.5 (120)		
4.5	5.0	50.4 (172)		
3.5	3.9	29.6 (88)		
2.5	2.7	0.0 (229)		

AQUILEGIA CAERULEA (BK - Tris/PEG Media); N = 30

<u>Initial pH</u>	<u>Final pH</u>	<u>% Germination</u>	Test	
			<u>Statistic</u>	<u>p</u>
6.5	6.3	57.9 (175)	102.71	.001
5.5	5.7	66.5 (176)		
4.5	4.8	69.4 (177)		
3.5	3.6	1.4 (157)		
2.5	2.6	0.0 (130)		

TABLE 3. Comparison of reproductive output in Erythronium fruits from natural pollinations in nonsprayed plants and from hand pollinations of flowers sprayed with acid rain simulant (pH 3.5) or ambient rain simulant (pH 5.6). Means were compared with the Mann-Whitney U test; means with different letters are significantly different at $p < .05$ level.

ERYTHRONIUM GRANDIFLORUM

	<u>Ambient Spray</u>	<u>Acid Spray</u>	<u>Non-Spray Control</u>
# SEEDS	Y = 37.13 a	Y = 39.27 a	Y = 32.49 b
PER FRUIT	s = 18.24	s = 20.68	s = 13.68
	n = 23	n = 22	n = 39
TOTAL WEIGHT	Y = 137.9 a	Y = 145.4 a	Y = 151.3 b
OF SEEDS PER	s = 69.9	s = 86	s = 83
FRUIT (mg)	n = 23	n = 22	n = 39
MEAN SEED	Y = 3.72 a	Y = 3.76 a	Y = 4.69 b
WEIGHT (mg)	s = 0.87	s = 1.15	s = 1.50
	n = 23	n = 22	n = 39

TABLE 4. Comparison of reproductive output in Aquilegia fruits from natural pollinations in nonsprayed plants and from hand pollinations of flowers sprayed with acid rain simulant (pH 3.5) or ambient rain simulant (pH 5.6). Means were compared with the Mann-Whitney U test; means with different letters are significantly different at $p < .05$ level.

AQUILEGIA CAERULEA

	<u>Ambient Spray</u>	<u>Acid Spray</u>	<u>Non-Spray Control</u>
# SEEDS	Y = 60.70 a	Y = 36.33 b	Y = 67.88 c
PER FRUIT	s = 44.65	s = 54.73	s = 52.71
	n = 10	n = 12	n = 68
TOTAL WEIGHT	Y = 48.2 a	Y = 20.5 b	Y = 41.0 c
OF SEEDS PER	s = 39.3	s = 27.5	s = 29.7
FRUIT (mg)	n = 10	n = 12	n = 68
MEAN SEED	Y = 0.70 a	Y = 0.58 b	Y = 0.63 c
WEIGHT (mg)	s = 0.10	s = 0.13	s = 0.24
	n = 10	n = 12	n = 68
% UNFILLED	Y = 34.3 a	Y = 57.5 b	Y = 54.5 b
SEEDS	s = 24.3	s = 32.2	s = 26.2
	n = 10	n = 12	n = 68

TABLE 6. Comparison of number of seeds per fruit and percentage of unfilled (aborted or unfertilized) seeds in Aquilegia fruits from hand pollinations of flowers sprayed with acid rain simulant (pH 3.5) or ambient rain simulant (pH 5.6) in five crosses between the same seed parent (S.P.) and pollen parent (P.P.).

AQUILEGIA CAERULEA (MATCHED CROSSES)

SEEDS/FRUIT

Cross #	Plant Number		Spray Treatment	
	<u>S.P.</u>	<u>P.P.</u>	<u>Ambient</u>	<u>Acid</u>
1.	39	3	42	16
2.	39	3	52	35
3.	1	35	53	2
4.	4	28	98	56
5.	10	26	16	6

% UNFILLED SEEDS

Cross #	Plant Number		Spray Treatment	
	<u>S.P.</u>	<u>P.P.</u>	<u>Ambient</u>	<u>Acid</u>
1.	39	3	41	65
2.	39	3	36	73
3.	1	35	41	93
4.	4	28	26	34
5.	10	26	59	85